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A TECHNIQUE FOR THE FLUORESCENCE STAINING OF FUNGAL NUCLEI

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Abstract

The fluorochrome "Hoechst 33258" provides a rapid and specific stain for fungal nuclei.

Uittreksel

'N TEGNIEK OM DIE KERNE VAN FUNGI FLUORISEEREND TE KLEUR

Die fluorochrome „Hoechst 33258" is 'n vinnige en spesifieke kleur vir die kerne van fungi.

Introduction

The techniques involved in the study of fungal nuclei are frequently lengthy, and the nuclei often show only a poor affinity for the stains employed.

However, the development of techniques for chromosome fluorescence has offered new opportunities in the field. Lemke *et al.* (1975) developed a method based on the use of fluorescent Feulgen reagents which offered a clear visualisation of nuclei in a variety of fungi.

The present communication describes a more rapid technique based on the use of the direct, DNA-binding fluorochrome "Hoechst 33258". The compound is chemically a bibenzimidazole derivative: 2-[2-(4-hydroxyphenyl)-6-benzimidazol]-6-(1-methyl-4-piperazyl)-benzimidazol-trihydrochloride. The stain, which in fact binds preferentially to the AT-rich regions of DNA (Weisblum and Haenssler, 1974), was recently introduced as a stain for constitutive heterochromatin in mammalian chromosomes (Hillwig and Gropp, 1972), and has since found widespread application in chromosome fluorescence studies (Mogford, 1977, 1978a, b, 1979).

Material and Methods

Cultures of *Saccharomyces cerevisiae* Hansen were maintained on standard malt agar slopes. Clumps of cells were removed using a spatula, and fixed in 3:1 ethanol:glacial acetic acid overnight. The cells were then rinsed in distilled water, smeared on an albumenised coverslip and air-dried. The preparations were stained

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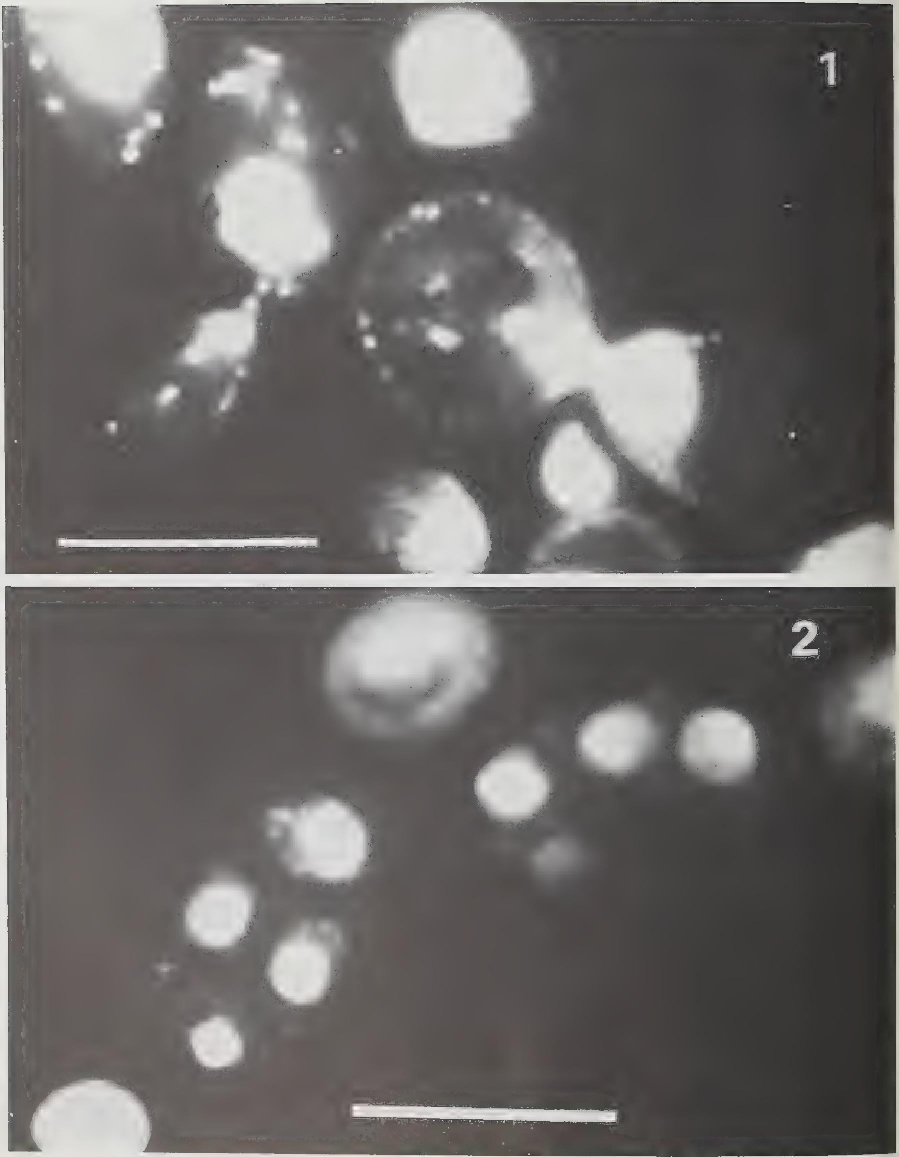

Figs 1–2.
Saccharomyces cerevisiae, stained as described in text. Fig. 1. Vegetative cells, budding. Fig. 2. Asci, showing nuclei within ascospores.

Bars represent 5 μ

in a 0,1 % ethanolic solution of Hoechst 33258 for 10 minutes at room temperature. The stained preparations were rinsed in ethanol, air-dried, mounted in 50 % glycerol and viewed using a Zeiss fluorescence microscope with exciter filter BG12 and barrier filter 50. Photographs were taken using Kodak Tri-X Pan film.

Differentiation of the nuclei was improved by storage of the completed preparations at 2 °C for periods of up to a fortnight.

Results and Discussion

The method produced a specific staining of the fungal nuclei, indicating their position within budding cells (Fig. 1) and ascospores (Fig. 2). Within vegetative cells, smaller fluorescent particles were also evident and presumably represented mitochondria or other DNA-containing organelles (Fig. 1).

It is clear that the method provides a rapid and effective alternative to conventional techniques. Its particular advantage is the removal of the hydrolysis stage necessary with most other methods. The further application of the method should yield valuable results.

References

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